

application. Applicants request a five-month extension of time pursuant to 37 C.F.R. §1.136(a) and enclose the required fee under 37 C.F.R. §1.17(a)(5). Applicants submit herewith a Substitute Sequence Listing in paper and computer readable form. Please consider the following amendments and remarks.

IN THE SPECIFICATION

Please delete the Sequence Listing ~~and~~ substitute therefor, the Substitute Sequence Listing included herewith.

Please **amend** the text beginning at page 19, line 31 and ending at page 20, line 5 with the following rewritten text:

αMSHR Forward Primer 1: (5'-TGT AAA ACG ACG GCC AGT RGT GCC TGG AGG TGT CCA T-3')(Sequence ID No. 1)

αMSHR Reverse Primer 5: (5'-CGC CCA GAT GGC CGC GAT GGA CCG-3')(Sequence ID No. 2); or

αMSHR Forward Primer 2: (5'-CGG CCA TCT GGG CGG GCA GCG TGC -3')(Sequence ID No. 3)

αMSHR Reverse Primer 2: (5'-GGA AGG CGT AGA TGA GGG GGT CCA-3')(Sequence ID No. 4); or

αMSHR Forward Primer 3: (5'-GCA CAT CGC CCG GCT CCA CAA GAC-3')(Sequence ID No. 5)

αMSHR Reverse Primer 3: (5'-GGG GCA GAG GAC GAC GAG GGA GAG-3')(Sequence ID No. 6).

Please **amend** the paragraph beginning at page 24, line 8 and ending at page 24, line 8 with the following rewritten text:

LA93 5' – GAGCAGCCCCTACCCCGGAATGCCAGTTGA – 3' (Sequence ID No. 7)

Please **amend** the paragraph beginning at page 24, line 10 and ending at page 24, line 11 with the following rewritten text:

b3 KIT56 5' - CTTTAAACAGAACATAAAAGCGGAAACATCATGCGAAGG - 3' (Sequence ID No. 8)

Please **amend** the paragraph beginning at page 25, line 2 and ending at page 25, line 6 with the following rewritten text:

b4 Figure 1. Partial nucleotide sequence (a) (Sequence ID Nos. 37-42) and the derived amino acid sequence (b) (Sequence ID Nos. 43-47) of the porcine aMSH-R gene as determined from a number of pig breeds. Position numbers for the nucleotide sequence are based upon nucleotide 1 being the A of the ATG initiation codon. Numbers of the amino acids are in accordance with the bovine BDF3 sequence (Vanetti *et al.*, FEBS Lett. **348**: 268-272 (1995)) to allow comparison.

Please **amend** the text beginning at page 27, line 1 and ending at page 27, line 3 with the following rewritten text:

b5 Figure 10: Nucleotide sequence of the porcine *KIT* cDNA from an animal of the Hampshire breed (SEQ ID NO. 47). The sequence is numbered with the first nucleotide of the N terminal methionine codon taken as 1.

Please **amend** the text beginning at page 27, line 13 and ending at page 27, line 18 with the following rewritten text:

b6 Nucleotide sequence of the 3' end of the porcine α MSHR coding region and adjacent 3' untranslated region. The TGA stop codon is highlighted in bold, the promoter binding sites for EPIG14 is shown in italics. Numbering is based on the system used in figure 1a in which nucleotide 1 is the A of the ATG initiation codon of the Wild Boar sequence. Bases in common with the European Wild Boar are marked with a dash. Missing bases are marked with a :. The

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Wild Boar sequence corresponds to SEQ ID NO. 49. The Meishan and Large Black sequences correspond to SEQ ID NO. 50. The Hampshire, Pietrain, and Duroc sequences correspond to SEQ ID NO. 51.

Please **amend** the text beginning at page 28, line 27 and ending at page 29, line 22 with the following rewritten text:

Primers MSHR Forward Primer 1: (5'-TGT AAA ACG ACG GCC AGT RGT GCC TGG AGG TGT CCA T-3')(Sequence ID No. 1); and

MSHR Reverse Primer 5: (5'-CGC CCA GAT GGC CGC GAT GGA CCG-3')(Sequence ID No. 2)

amplify a 428 bp fragment from the 5' half of the gene.

bn
Primers MSHR Forward Primer 2: (5'-CGG CCA TCT GGG CGG GCA GCG TGC - 3')(Sequence ID No. 3);

and α MSHR Reverse Primer 2: (5'-GGA AGG CGT AGA TGA GGG GGT CCA-3')(Sequence ID No. 4)

amplify a 405 bp fragment of the 3' half of the gene.

As these two fragments are non-overlapping a third primer pair

αMSHR Forward Primer 4 (5'-TGC GCT ACC ACA GCA TCG TGA CCC TGC-3')(Sequence ID No. 10); and

by cont
αMSHR Reverse Primer 4 (5'-GTA GTA GGC GAT GAA GAG CGT GCT-3')(Sequence ID No. 11)

were used to amplify a 98 bp fragment which spans the 50 bp gap. PCR was carried out on a DNA thermal cycler (Perkin Elmer 9600) in a total volume of 20 µl containing 25 ng genomic DNA, 1.0 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 200 µM dNTPs, 0.5 U AmpliTaq Gold (Perkin Elmer) and 10 pmol of both KIT21 and KIT35 primers. To activate AmpliTaq Gold, initial heat denaturation was carried out at 94° C for 10 minutes followed by 32 cycles each consisting of 45 sec at 94° C, 45 sec at 53° C and 45 sec at 72° C. The final extension lasted for 7 min at 72° C. PCR products were cloned into vector pUC18 using the SureClone ligation kit (Pharmacia Biotech).

Please **amend** the paragraphs beginning at page 31, line 14 and ending at page 31, line 17 with the following rewritten paragraphs:

B8
MSHR Forward Primer 3 sequence: 5'-GCA CAT CGC CCG GCT CCA CAA GAC-3'

(Sequence ID No. 5)

MSHR Reverse Primer 3 sequence : 5'-GGG GCA GAG GAC GAC GAG GGA GAG-3')(Sequence ID No. 6)

Please **amend** the paragraph beginning at page 37, line 11 and ending at page 37, line 12 with the following rewritten paragraph:

Forward primer sequence: 5'-CTG CCT GGC CGT GTC GGA CCT G-3' (Sequence ID No. 12)

Reverse primer sequence: 5'-CTG TGG TAG CGC AGC GCG TAG AAG-3' (Sequence ID No. 13).

Please **amend** the paragraph beginning at page 39, line 8 and ending at page 39, line 9 with the following rewritten paragraph:

5'-TGAGGTAGGAGAGTTTTGGG-3' (Sequence ID No. 14)

5'-TCGAAATTGAGGGGAAGACC-3' (Sequence ID No. 15)

Please **amend** the paragraph beginning at page 40, line 13 and ending at page 40, line 28 with the following rewritten paragraph:

First-strand cDNA synthesis was accomplished using the First-Strand cDNA Synthesis kit (Pharmacia Biotech) so that ~100 ng mRNA was randomly primed by 0.1 μ g pd(N6) in a total volume of 15 μ l. Two μ l of the completed first cDNA strand reaction was then directly used per 12 μ l PCR reaction by adding 10 μ l PCR mix containing 10 pmol each of the mouse/human derived primers KIT1F and KIT7R (5'-TCR TAC ATA GAA AGA GAY GTG ACT C-3' (Sequence ID No. 28) and 5'-AGC CTT CCT TGA TCA TCT TGT AG-3' (Sequence ID No. 29), respectively; Moller et al. 1996, *supra*), 1.2 μ l 10 x PCR-buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl) and 0.5U of AmpliTaq polymerase (Perkin-Elmer) incubated with an equal amount Taqstart antibody (Clontech) at 25° C for 5 min to achieve a hot start PCR. The reaction was covered with 20 μ l mineral oil and thermocycled in a Hybaid Touchdown machine (Hybaid) with 40 cycles at 94° C for 1 min, 55-48° C (touchdown one degree per cycle the first seven cycles and then 48° C in the remaining cycles) for 1 min and 72° C for 1 min. After PCR 2 μ l loading dye was added to each sample which were then loaded on 4% agarose gel (Nusieve/Seakem 3:1, FMC Bioproducts) and electrophoresed with 100V for 80 min. Products were visualized by ethidium bromide staining and UV-illumination.

Please **amend** the paragraph beginning at page 42, line 2 and ending at page 42,
line 12 with the following rewritten paragraph:

B12
A 175 bp region including the boundary between exon 17 and intron 17 of the *KIT* gene was amplified for sequence analysis using forward primer KIT21 (5' – GTA TTC ACA GAG ACT TGG CGG C – 3')(Sequence ID No. 16) and reverse primer KIT35 (5' – AAA CCT GCA AGG AAA ATC CTT CAC GG -3')(Sequence ID No. 17). PCR was carried out on a DNA thermal cycler (Perkin Elmer 9600) in a total volume of 20 µl containing 25 ng genomic DNA, 1.0 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 200 µM dNTPs, 0.5 U AmpliTaq Gold (Perkin Elmer) and 10 pmol of both KIT21 and KIT35 primers. To activate AmpliTaq Gold, initial heat denaturation was carried out at 94° C for 10 minutes followed by 32 cycles each consisting of 45 sec at 94° C, 45 sec at 55° C and 45 sec at 72° C. The final extension lasted for 7 min at 72° C. PCR products were cloned into vector pUC18 using the SureClone ligation kit (Pharmacia Biotech).

Please **amend** the paragraph beginning at page 49, line 24 and ending at page 49,
line 25 with the following rewritten paragraph:

B13
Forward GAATATTGTTGCTATGGTGATCTCC *KIT1*-FOR (Sequence ID No. 18)
Reverse CCGCTTCTGCGTGATCTTCCTG *KIT1*-REV (Sequence ID No. 19)

Please **amend** the paragraph beginning at page 49, line 29 and ending at page 49,
line 30 with the following rewritten paragraph:

B14
Forward CTGGATGTCCTGTGTTCCCTGT *CRC*-FORWARD (Sequence ID No. 20)
Reverse AGGTTTGTCTGCAGCAGAAGCTC *CRC*-REVERSE (Sequence ID No. 21)

Please **amend** the paragraph beginning at page 53, line 15 and ending at page 53,
line 17 with the following rewritten paragraph:

Forward GAAAGTGA(C/T)GTCTGGTCCTAT(C/G)GGAT *KIT*DEL2-FOR (Sequence
ID No. 22)

Reverse AGCCTTCCTTGATCATCTTGTAG *KIT*DEL2-REV (Sequence ID No. 23)

Please **amend** the paragraph beginning at page 56, line 28 and ending at page 56,
line 29 with the following rewritten paragraph:

forward TGTGGGAGCTCTTCTCTTTAGG *KIT*DEL1-FOR (Sequence ID No. 24)

reverse CCAGCAGGACAATGGGAACATCT *KIT*DEL1-REV (Sequence ID No. 25)

Please **amend** the paragraph beginning at page 58, line 17 and ending at page 59,
line 5 with the following rewritten paragraph:

mRNA was isolated from peripheral blood leukocytes from white (Landrace/Large White) and coloured (Hampshire) pigs using the Message Maker mRNA isolation system (Gibco BRL) with one mRNA selection from total RNA. 100 ng poly(A)+ mRNA was reverse-transcribed with random primers (First-Strand cDNA Synthesis kit, Pharmacia Biotech) and the product was used at a 1:10 dilution for RT-PCR using the proof-reading Advantage KlenTaq Polymerase (Clontech) according to the manufacturer's recommendation. The following primers were used to amplify almost the entire coding sequence and some of the 5' untranslated region: KIT40 (5' – GGC TCT GGG GGC TCG GCT TTG C – 3')(Sequence ID No. 26) corresponding to the untranslated region and KIT22S (5' – TCA GAC ATC TTC GTG GAC AAG CAG AGG – 3')(Sequence ID No. 27) corresponding to exon 21; both primers had been designed using consensus sequence of the human and mouse *KIT* sequences in the GENE BANK database. The RT-PCR products were gel purified and cloned using the pGEM-T vector system (Promega). Plasmid clones were sequenced using a set of internal primers and the ABI Prism™ dRhodamine Terminator Cycle Sequencing Kit (PE Applied Biosystems). Two subclones representing each type of *KIT* sequence were initially sequenced and in those cases where a discrepancy was observed (possibly due to PCR errors) additional clones were sequenced over those particular

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nucleotide sites. RT-PCR analysis of *KIT* exon 16-19 was carried out with the primers KIT1F (5' – TCR TAC ATA GAA AGA GAY GTG ACT C – 3')(Sequence ID No. 28) and KIT7R (5' – AGC CTT CCT TGA TCA TCT TGT AG – 3')(Sequence ID No. 29).

Please **amend** the paragraph beginning at page 63, line 10 and ending at page 63, line 26 with the following rewritten paragraph:

B.18
Allelic discrimination reactions were set up using the PE Applied Biosystems TaqMan™ system. 25 µl reactions contained the primers E19FOR (5' – GAGCAGCCCCTACCCCGGAATGCCAGTTGA – 3')(Sequence ID No. 30) and E19REV (5' – CTTTAAAACAGAACATAAAAGCGGAAACATCATGCGAAGG – 3')(Sequence ID No. 31) at 300 nM, 8% glycerol (w/v) 1X TaqMan™ buffer A (PE Applied Biosystems), 5 mM MgCl₂, 200 µM dATP, dGTP, dCTP, and dUTP, 0.65 units AmpliTaq Gold™ (PE Applied Biosystems), 0.25 units AmpErase™ UNG (PE Applied Biosystems) and the TaqMan™ probes E19PC (5' – CATACATTTCCGCAGGTGCATGC – FAM)(Sequence ID No. 52) and E19PT (5' – TCATACATTTCCACAGGTGCATGC – TET)(Sequence ID No. 53) at a concentration of 100 mM. 1 µl of crude lysate DNA was used as template. PCR amplification was carried out using a PE9600 thermal cycler (PE Applied Biosystems) or the ABI7700 Prism (PE Applied Biosystems) with a thermal cycling regime of 50° C for 2 min followed by 95° C for 10 min followed by 40 cycles of 95° C 15 sec, 62° C 1 min. 8 control samples of each homozygote genotype, 2678C and 2678T, and 8 no template controls where deionized water was substituted for template controls were used per 96 well plate. Allele identification based on these reactions was carried out using the allelic discrimination function of the ABI7700 Prism (PE Applied Biosystems).

Please **amend** the paragraph beginning at page 66, line 30 and ending at page 66, line 31 with the following rewritten paragraph:

B.19
EPIG10 5' – GGT CTA GAT CAC CAG GAG CAC TGC AGC ACC - 3' (Sequence ID No. 32)

EP1G16 5' – GGG AAG CTT GAC CCC CGA GAG CGA CGC GCC - 3' (Sequence ID
No. 33)

Please **amend** the paragraphs beginning at page 68, line 22 and ending at page 68,
line 34 with the following rewritten paragraphs:

| | | | | | |
|-----------|------------|------------|------------|-------------------|------------|
| Pietrain | CGACGCGCCC | TCCCTGCTCC | CTGGCGGGAC | GATGCCTGTG | CTTGGCCCGG |
| Meishan | ----- | ----- | ----- | ----- | ----- |
| Wild Boar | ----- | ----- | ----- | ----- | ----- |
| | | | | | |
| Pietrain | AGAGGAGGCT | GCTGGCTTCC | CTCAGCTCCG | CGCCCCCAGC | CGCCCCCCCC |
| Meishan | ----- | ----- | ----- | ----- | ----- |
| Wild Boar | ----- | ----- | ----- | ----- | ----- |
| | | | | | |
| Pietrain | GCCTCGGGCT | GGCCGCCAAC | CAGACCAACC | AGACGGGCCC | CCAGTGCCTG |
| Meishan | ----- | ----- | ----- | ----- | ----- |
| Wild Boar | ----- | ----- | ----- | ----- | ----- |
| | | | | | |
| Pietrain | GAGGTGTCCA | TT | | | |
| Meishan | ----- | -- | | | |
| Wild Boar | ----- | -- | | | |

These results are also incorporated into figure 1a. The italicized nucleotides of the Pietrain sequence correspond to nucleotides 22 to 30 of EP1G16 (Sequence ID No. 33). The remaining Pietrain sequence shown corresponds to nucleotides 8 to 160 of SEQ ID NO. 39. The Meishan sequence shown corresponds to nucleotides 8 to 158 of SEQ ID NO. 38. The Wild Boar sequence shown corresponds to nucleotides 8 to 158 of SEQ ID NO. 37.

Please **amend** the paragraph beginning at page 69, line 8 and ending at page 69,
line 9 with the following rewritten paragraph:

MC1R121A 5' – Hex – GGA CTC CAT GGA GCC GCA GAT GAG CAC GGT - 3'
(Sequence ID No. 34)